

but quite condensed and its occasional mitotic figures were usually abortive.

The fetuses of Group C were operated and then given 0.25 to 0.5 mg adrenotrophin, in 4 to 5 divided doses, and were killed 49 to 54 hours after the first injection. Microscopically, the adrenal cortex resembled that of the fetuses of Group A. The relative volume of the adrenal, using operated littermates as controls, was 1.01 to 3.21, the theoretical median being 2.35. Also, the adrenals were much larger than those of normal littermates of the same sex (relative volume, up to 2.29).

In considering all aspects of the study, the

data show that injected hypophyseal adrenotrophin causes the fetal adrenal to grow more rapidly than in controls. They suggest that removal of the fetal head (hypophysis) inhibits growth of the adrenal and causes abnormal changes in the cortical cells. They indicate that this inhibition and these changes may be prevented by giving a series of injections of adrenotrophin.

In conclusion, these observations may be taken as experimental evidence of the production of adrenotrophin by the hypophysis of the fetal rat.

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Effect of Sulfamethazine on the Testes and Accessory Glands of Normal and Hypophysectomized Rats.

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Asplin and Boyland¹ have reported that chronic administration of sulfamethazine (4,6-dimethyl-2-sulfanilamidopyrimidine) causes a hyperplasia and increase in the weight of the testes and some increase of weight of the seminal vesicles and prostate in male rats receiving the drug from birth to a weight of about 50 g. In older males an increase in weight of only the seminal vesicles and the prostate was noted. They found that the drug had no effect on the seminal vesicles and prostate of castrated rats and postulated that sulfamethazine causes hyperplasia of the seminal vesicles and the prostate by stimulation of the testes.

Since the accessory glands of male rats are normally maintained and stimulated by androgen secreted by the interstitial tissue of the testes under the influence of the anterior pituitary gland, it seemed of interest to investigate whether the effects reported by Asplin and Boyland in young rats could be

secondary to the stimulating effect of the drug on the anterior pituitary gland. If that were the case, the ablation of the anterior pituitary gland would nullify the reported hypertrophy of the seminal vesicles and prostate in rats receiving sulfamethazine.

Experimental. Forty-one immature rats of weaning age (21-day old) of the same inbred stock were divided into 2 groups of 20 and 21 each. The hypophysis of the rats in the first group* was removed by the parapharyngeal approach according to the technic of Smith.² Half of the rats in both the hypophysectomized and the unoperated groups was placed on a stock laboratory diet. The other half of each group was fed on a similar diet to which 0.2% by weight of sulfamethazine† was added. The rats were weighed at the beginning of the ex-

* Four rats in this group died within one week after hypophysectomy.

² Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

¹ Asplin, F. D., and Boyland, E., *Brit. J. Pharm. and Chemother.*, 1947, **2**, 79.

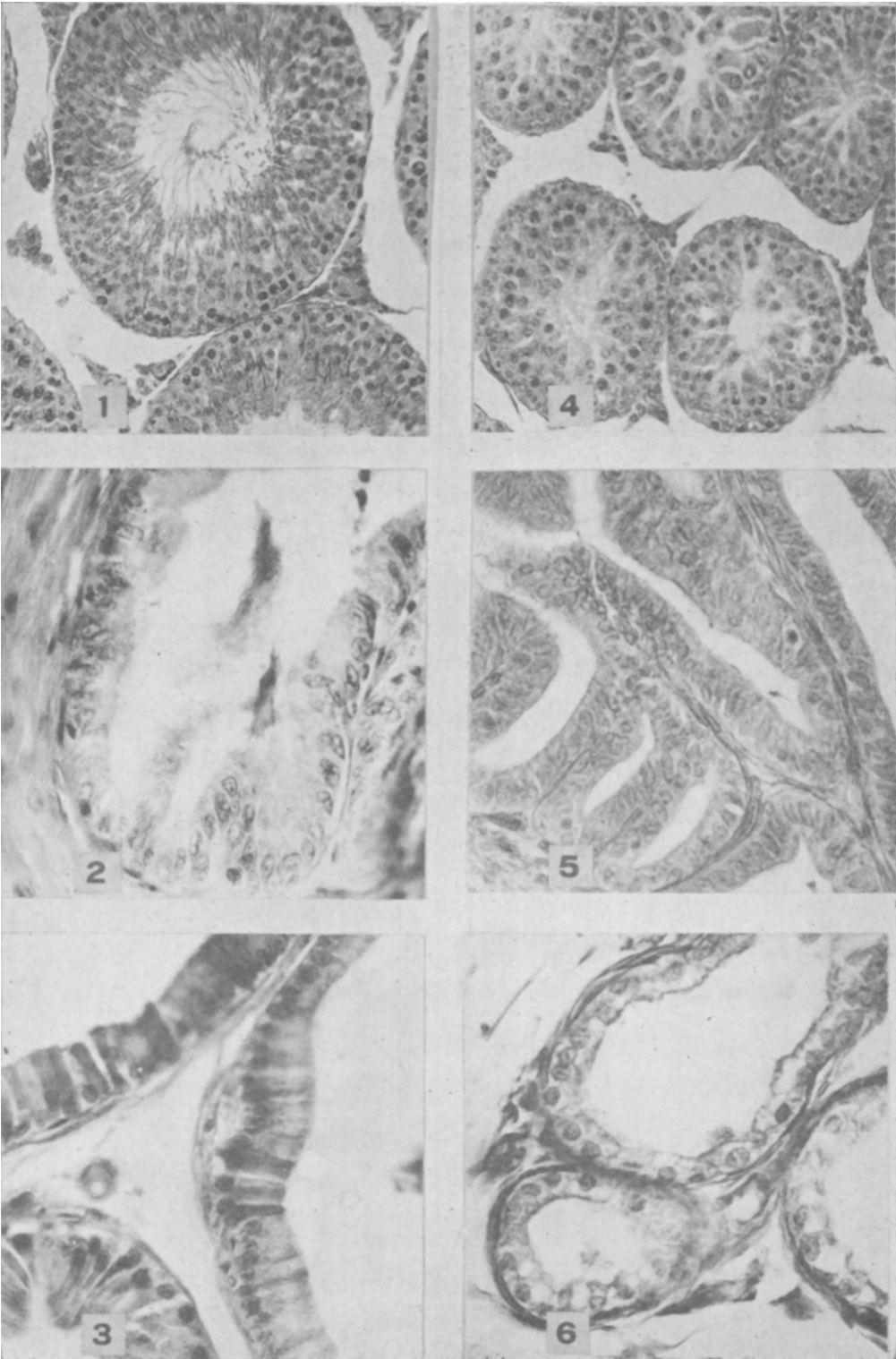
† Sulfamethazine was kindly furnished by Mr. W. A. Lott of the Squibb Institute for Medical Research.

TABLE I.
The Effect of a Diet Containing Sulfamethazine on the Weight of Organs of Normal and Hypophysectomized Rats.

No. of rats	Type	Treatment	Body wt, g			
			Start of exp. Mean and S.D.	P*	End of exp. Mean and S.D.	P
8	Hypx		50.4 ± 2.9	>0.05	60.7 ± 5.7	>0.05
8	"	Sulfameth.	47.5 ± 2.8		72.0 ± 10.8	
10	Normal		48.0 ± 8.6	>0.20	197.5 ± 25.4	<0.01
11	"	"	44.5 ± 2.6		177.8 ± 18.1	

Wt of organs per 100 g body weight									
No. of rats	Type	Treatment	Testes, mg		P*	Seminal vesicles, mg		Anterior prostate, mg	P.
			Mean	S.D.		Mean	S.D.	Mean	S.D.
8	Hypx		156.5 ± 45.1	<0.05		7.0 ± 1.3	7.3 ± 1.4	=0.2	
8	"	Sulfameth.	117.3 ± 19.9			6.2 ± 1.2	6.8 ± 1.0	=0.4	
10	Normal		1205.6 ± 97.1	=0.05		105.2 ± 31.4	67.5 ± 30.9	<0.01	
11	"	"	1052.0 ± 205.5			50.2 ± 26.9	35.4 ± 24.2	=0.02	

* P values (Fisher 1944) are those comparing the means of the groups in parentheses.



Rat No. 39700. Fed on stock laboratory diet, age 63 days at time of necropsy. Hematoxylin and eosin stains:

FIG. 1. Testes	× 200
FIG. 2. Seminal vesicle	× 400
FIG. 3. Anterior prostate	× 400

Rat No. 39829. Fed for 35 days on stock laboratory diet to which was added 0.2% of sulfamethazine, age 56 days at time of necropsy. Hematoxylin and eosin stains:

FIG. 4. Testes	× 200
FIG. 5. Seminal vesicle	× 400
FIG. 6. Anterior prostate	× 400

periment and twice weekly thereafter. To be sure of adequate absorption, the concentration of free sulfamethazine in the blood of all rats treated with the drug was determined at intervals according to the micromethod of Bratton and Marshall.³ All rats were killed with ether at the age of 56 to 63 days. The testes, seminal vesicles, anterior prostate, thyroid, adrenal and anterior pituitary glands of all the rats were carefully dissected, freed of connective tissue and weighed on a sensitive torsion balance. Bouin's fluid was used as fixative and paraffin sections were made and stained with hematoxylin and eosin.

Results. It will be seen from Table I that in normal rats, fed on diet containing sulfamethazine, the weight of the testes, seminal vesicles and anterior prostate per 100 g body weight were significantly smaller than those of rats fed on stock laboratory diet. The weights of the thyroid, adrenal and anterior pituitary glands (average 2.7 ± 0.5 mg for those of rats fed on stock laboratory diet and 2.8 ± 0.4 mg for those of rats fed on same diet to which 0.2% of sulfamethazine was added) of the normal rats fed on diet with or without sulfamethazine did not show any significant difference. A definite retardation of growth was noted in the normal rats dosed with sulfamethazine. In hypophysectomized rats, the testes, thyroid, and adrenals from animals receiving sulfamethazine were lighter than the same organs removed from control rats. The concentration of free sulfamethazine in the blood of both the normal and hypophysectomized rats receiving the drug varied from 5 to 13.5 mg per 100 cc of blood.

Microscopically, in the normal rats that were fed on diet containing sulfamethazine the most striking changes were seen in the testes

(Fig. 1 and 4). The tubules were much smaller in diameter than those of their litter-mate controls. There was complete absence of spermatogenic activities as evident by the total lack of spermatozoa. The interstitial tissue, however, did not seem to exhibit any pathological changes as compared with that in the testes of the untreated normal rats. The epithelial cells of the seminal vesicle in the treated rats showed a definite decrease in height. It will be seen in Fig. 2 and 5 that while the nuclei of the epithelial cells of the seminal vesicles of the untreated control rats occupy one-half or less of the cells, those of the epithelium of the sulfamethazine treated rats occupy two-thirds or more of the cells. Similar changes, although at places were not as marked as those seen in the seminal vesicle, were present in the epithelium of the anterior prostate gland. There were also found in the epithelium numerous vacuoles which are indicative of degenerative changes in the organ (Fig. 3 and 6). Although not accompanied by any significant change of weight, there were hyperplasia of the epithelium and vacuolation of the colloid in the acini of the thyroid of the normal rats receiving sulfamethazine in their diet. Many of the acini were obliterated. No significant change could be seen in the sections of the adrenal glands. In the hypophysectomized rats the testes of sulfamethazine-treated rats showed further degenerative changes, *i.e.* decrease in the number of germinal cells and increase of Sertoli cells in the germinal epithelium, compared with those of rats fed on stock laboratory diet. No important difference was noted in the sections of the seminal vesicles, anterior prostate, thyroid, or adrenal glands of the hypophysectomized rats fed on diet with or without sulfamethazine.

Discussion. It is evident from the foregoing data that contrary to the observations of

³ Bratton, C., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

Asplin and Boyland,¹ the chronic administration of sulfamethazine to young male rats caused atrophy of the testes and accessory glands. The basis for the discrepancy is not clear and it is not believed that the higher concentration of sulfamethazine in the diet (0.2% instead of 0.1%) of the experiments here reported accounts for the difference in results. Since atrophic changes of the testes were observed both grossly and microscopically in the present experiments in both the normal and hypophysectomized rats chronically fed with sulfamethazine, it would appear that the effect of the drug on the gonads of the young rats is one of direct inhibition. Although the interstitial tissue of the testes of the sulfamethazine-treated normal rats did not exhibit any histopathological changes, the atrophic changes in the accessory glands in these rats may still be considered to be secondary to a decreased secretory activity, without any detectable histological changes in the interstitial tissue.

Except that there was no significant change in weight (per 100 g body weight), the hyperplasia of the thyroid gland of the sulfamethazine-treated normal rats conformed with the observations of MacKenzie⁴ and Ast-

wood⁵ who have reported that sulfonamides cause hyperplasia of thyroid glands in rats provided that the pituitary is intact. It remains to be determined, however, what significance should be attached to the apparent decrease in weights of the thyroid and adrenal glands in the hypophysectomized rats treated with sulfamethazine.

Summary. Chronic administration of sulfamethazine to normal male rats causes gross and microscopic atrophic changes in the testes, seminal vesicles and anterior prostate but a hyperplasia of the thyroid gland. The reported hypertrophy of the accessory organs¹ of the young male rats treated with sulfamethazine was not confirmed. When hypophysectomized rats were fed with sulfamethazine, only increased atrophy of the testes was manifested. The atrophic changes that occurred in the testes and accessory organs in normal rats, as well as the similar changes in the testes of the hypophysectomized male rats after chronic administration of sulfamethazine, are attributed to a direct effect of the drug on the testes.

The author wishes to express his indebtedness to Dr. H. B. van Dyke for suggestions and advice in carrying out the experiments, and Dr. P. E. Smith in examining the histopathological sections.

⁴ MacKenzie, C. G., and MacKenzie, J. B., *Endocrinology*, 1943, **32**, 185.

⁵ Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R., *Endocrinology*, 1943, **32**, 210.

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Histochemical Variations in the Metrial Gland of the Rat During Pregnancy and Lactation.

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The metrial gland of the rat develops during pregnancy in association with the uterine blood vessels in the region of attachment of the mesometrium to the uterus. Some phases

of the histology of the metrial gland have been investigated.¹⁻⁴ Since little is known concerning the possible endocrine function of the

*The author wishes to express his appreciation to Miss Barbara Piper for her technical assistance.

¹ Goldmann, E. E., *Beitr. z. klin. Chir.*, 1912, **78**, 1.

² Gérard, P., *C. R. Soc. de Biol.*, 1925, **93**, 457.

³ Selye, H., and McKeown, T., *Proc. Roy. Soc., London, Series B*, 1935, **119**, 1.

⁴ Asplund, J., Borell, U., and Holmgren, H., *Z. f. mikr.-anat. Forsch.*, 1940, **48**, 478.